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L1: Entry 1 of 1 File: USPT Jul 4, 2000

DOCUMENT-IDENTIFIER: US 6084067 A
TITLE: CTLA4/CD28 ligands and uses therefor

BSPR:
Another embodiment of the invention provides antibodies, preferably monoclonal antibodies, specifically reactive with a peptide of a novel B lymphocyte antigen or fusion protein as described herein. Preferred antibodies are anti-human B7-2 monoclonal antibodies produced by hybridoma cells HF2.3D1, HA5.2B7 and HA3.1F9. These hybridoma cells have been deposited with the American Type Culture Collection at ATCC Accession No. HB11686 (HF2.3D1), ATCC Accession No. HB 11687 (HA5.2B7), and ATCC Accession No. HB11688 (HA3.1F9).

DRPR:
FIGS. 19A-19C depict flow cytometric profiles of cells stained with an anti-hB7-2 monoclonal antibody, HF2.3D1. Cells stained with the antibody were CHO cells transfected to express human B7-2 (CHO-hB7.2), NIH 3T3 cells transfected to express human B7-2 (3T3-hB7.2) and control transfected NIH 3T3 cells (3T3-neo). The anti-hB7.2 antibody B70 was used as a positive control.

DEPR:
Particularly preferred antibodies are anti-human B7-2 monoclonal antibodies produced by hybridomas HA3.1F9, HA5.2B7 and HF2.3D1. The preparation and characterization of these antibodies is described in detail in Example 8. Monoclonal antibody HA3.1F9 was determined to be of the IgG1 isotype; monoclonal antibody HA5.2B7 was determined to be of the IgG2b isotype; and monoclonal antibody HF2.3D1 was determined to be of the IgG2a isotype. Hybidoma cells were deposited with the American Type Culture Collection, which meets the requirements of the Budapest Treaty, on Jul. 19, 1994 as ATCC Accession No. HB11688(hybridoma HA3.1F9), ATCC Accession No. HB11687(HA5.2B7) and ATCC Accession No. HB11686(HF2.3D1).

DEPR:
Supernatants from the hybridomas HA3.1F9, HA5.2B7 and HF2.3D1 were further characterized by competitive ELISA, in which the ability of the monoclonal antibodies to inhibit the binding of biotinylated hCTLA4Ig to immobilized hB7-2 immunoglobulin fusion proteins was examined. Biotinylation of hCTLA4Ig was performed using Pierce Immunopure NHS-LC Biotin (Cat. No. 21335). B7-2 immunoglobulin fusion proteins used were: hB7.2-Ig (full-length hB7-2), hB7.2-VIg (hB7-2 variable domain only) and hB7.2-CIg (B7-2 constant domain only). A hB7.1 -Ig fusion protein was used as a control. For the ELISA, 96 well plates were coated with the Ig fusion protein (50 .mu.l/well of a 20 .mu.g/ml solution) overnight at room temperature. The wells were washed three times with PBS, blocked with 10% fetal bovine serum (FBS), 0.1% bovine serum albumin (BSA) in PBS for 1 hour at room temperature, and washed again three times with PBS. To each well was added 50 .mu.I of Bio-hCTLA4-Ig (70 ng/ml) and 50 .mu.l of competitor monoclonal antibody supernatant. Control antibodies were an anti-B7.1 mAb (EW3.5D12) and the anti-hB7-2 mAb B70 (IgG2bi.kappa., obtained from Pharmingen). The wells were washed again and streptavidin-conjugated horse radish peroxidase (from Pierce, Cat. No. 21126; 1:2000 dilution, 50 .mu.l/well) was added and incubated for 30 minutes at room temperature. The wells were washed again, followed by a 30 minute incubation in 50 .mu.l per well of ABTS in 0.1 M Na-Citrate, pH 4.2 to which a 1:1000

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File 410:Chronolog(R)  1981-2000 Jul/Aug
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            210  HF2
            148  3D1
              1  HF2(W)3D1
          13085  B7
        7418396  2
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      S1       1  (3D1 OR HF2(W)3D1) AND B7(W)2
? t s1/3/all


 1/3/1     (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0177703 DBA Accession No.: 95-04524 PATENT
Nucleic acids encoding CTLA4/CD28 counter receptor, **B7-2** - specified DNA sequence; expression in host cell culture, tumor cell and in transgenic mouse; and specific monoclonal antibody produced by a hybridoma

AUTHOR: Freeman G J; Nadler L M; Gray G S; Greenfield E
PATENT ASSIGNEE: Dana-Farber-Cancer-Inst.; Repligen 1995

PATENT NUMBER: WO 9503408 PATENT DATE: 950202 WPI ACCESSION NO.: 95-075236 (9510)
PRIORITY APPLIC. NO.: US 147773 APPLIC. DATE: 931103
NATIONAL APPLIC. NO.: WO 94US8423 APPLIC. DATE: 940726
LANGUAGE: English